A substituted dextran enhances muscle fiber survival and regeneration in ischemic and denervated rat EDL muscle

PASCAL DESGRANGES,[1] CHRISTEL BARBAUD, JEAN-PIERRE CARUELLE, DENIS BARRITAULT,[2] AND JEAN GAUTRON

Laboratoire de Recherche sur la Croissance Cellulaire, la Régénération et la Réparation Tissulaires, Université Paris XII-Val de Marne, France UPRESA - CNRS 7053.

ABSTRACT **Ischemia and denervation of EDL muscle of adult rat induce a large central zone of degeneration surrounded by a thin zone of peripheral surviving muscle fibers. Muscle regeneration is a complex phenomenon in which many agents interact, such as growth factors and heparan sulfate components of the extracellular matrix. We have shown that synthetic polymers, called RGTA (as regenerating agents), which imitate the heparan sulfates, are able to stimulate tissue repair when applied at the site of injury. In crushed muscles, RGTA were found to accelerate both regeneration and reinnervation. *In vitro*, RGTA act as protectors and potentiators of various heparin binding growth factors (HBGF). It was postulated that *in vivo* their tissue repair properties were due in part to an increase of bioavailability of endogenously released HBGF. In the present work, we show that ischemic and denervated EDL muscle treated by a unique injection of RGTA differs from the control after 1 wk in several aspects: *1*) the epimysial postinflammatory reaction is inhibited and the area of fibrotic tissue among fibers is reduced; *2*) the peripheral zone, as measured by the number of intact muscle fibers, was increased by more than twofold; and *3*) In the central zone, RGTA enhances the regeneration of the muscle fibers as well as muscle revascularization. These results suggest that RGTA both protects muscle fibers from degeneration and preserves the differentiated state of the surviving fibers. For the first time it is demonstrated that a functionalized polymeric compound can prevent some of the damage resulting from muscle ischemia. RGTA may therefore open a new therapeutic approach for muscle fibrosis and other postischemic muscle pathologies.—Desgranges, P., Barbaud, C., Caruelle, J.-P., Barritaoult, D., Gautron, J. A substituted dextran enhances muscle fiber survival and regeneration in ischemic and denervated rat EDL muscle. *FASEB J.* 13, 761–766 (1999)**

Key Words: skeletal muscle · RGTA · ischemia

ONE OF THE fundamental properties of skeletal muscle is its ability to regenerate after damage caused by injury or disease (1, 2). Among these forms of damage, ischemia and denervation after free whole skeletal muscle transplantation induce characteristic phases of degenerative and regenerative changes (1, 3). During the first week, transplanted extensor digitorum longus (EDL)[3] in rat exhibits a large central zone of necrotic muscle fibers, surrounded by a thin peripheral layer of surviving muscle fibers. Muscle regeneration is associated with revascularization and reinnervation, and occurs during 60 to 90 days after transplantation (4).

Regeneration of skeletal muscle is a natural process in adults that differs in two aspects from muscle differentiation observed during fetal development. First, regeneration is preceded by a more or less complete fiber myolysis, inducing activation of preexisting satellite cells (5). Myolysis results from the action of endogenous proteases such as calpains (6) activated during the inflammatory process. Second, the regenerating process takes place within the remnants of the original muscle basal lamina. The basal lamina may be completely degraded; subsequent regeneration leads to changes in muscle structure, which is less efficient (7). Hence, proper muscle regeneration depends on extracellular components and appropriate growth factors that will trigger satellite cells to proliferate, migrate, and form new fibers (8, 9).

In previous work we have shown that some synthetic polymers that mimic the action of heparan sulfate, called RGTA (for regenerating agents), were able to stimulate tissue repair when applied at the

[1] Present address: Hospital Henri Mondor - Department of Vascular Surgery. 51, Ave du Maréchal de Lattre de Tassigny, 94010 Créteil - France.

[2] Correspondence: E-mail: barritault@univ-paris12.fr

[3] Abbreviations: EDL, extensor digitorum longus; FGF, fibroblast growth factors; HBGF, heparin binding growth factors; IGF, insulin-like growth factor; PBS, phosphate-buffered saline; RGTA, regenerating agents; TGF, tumor growth factor.

0892-6638/99/0013-0761/$02.25 © FASEB

site of injury (10). Indeed, RGTA was able to enhance skin (10), bone (11), colonic (12), and corneal healing (13). The RGTA used was a dextran derivative containing defined amounts of substituted carboxymethyl, benzylamide, and sulfonate groups.

In vitro, this RGTA interacts with and protect various heparin binding growth factors (HBGF) such as fibroblast growth factor(s) [FGF(s)] or tumor growth factor β (TGF-β) against proteolytic degradation and thus enhances their bioavailability (14, 16). This molecule is devoid of anticoagulant activity and was found to inhibit several proteases (17, 18). RGTA could also accelerate regeneration and reinnervation of crushed muscles. We have shown that a single injection of RGTA, just after injury, was able to stimulate the regeneration and differentiation of muscle fibers. After denervation, this treatment also enhanced reformation of the motor end-plates on the regenerated muscle fibers (19–21).

Considering the importance of peripheral muscle ischemia-induced degeneration in human clinics, we investigated the effect of RGTA on muscle regeneration in a modified model of ischemic and denervated EDL rat muscle.

MATERIALS AND METHODS

1) RGTA

RGTA was a carboxymethyl benzylamide sulfonate dextran synthesized from native dextran T40 (MW 40,000) batch 32202 (Pharmacia Fine Chemicals, Uppsala, Sweden), as described by Mauzac and Josefonvicz (22) by a sequential substitution of three addition steps on glucose residues: carboxymethylation, followed by coupling benzylamide and finally sulfonation.

The chemical composition was determined by microanalysis and spectrophotometric techniques. Their degree of substitution by addition of the various reactive groups can be controlled, yielding a large family of different compounds. For the RGTA-11 used in these experiments, the percentage of hydroxyl groups bearing substitutions was 110% for methylcarboxylic acid, 2.5% for benzylamide, and 36.5% for benzylamide sulfonate groups respectively according to Mauzac determination. It was selected for its low anticomplement activity, its very low anticoagulant activity (4 IU/mg), and its ability to mimic heparin or heparan sulfate proteoglycans in their *in vitro* interactions with FGFs.

2) Ischemic muscle model

The ischemic muscle model was derived from that developed by Carlson (3) on the EDL of adult rat. Two-month-old male Wistar rats (n=15) weighting 175-200 g (from IFFA-CREDO, Lyons, France) were used. All procedures complied with the 'Principles of Laboratory Animal Care' and 'Guide for the Care and Use of Laboratory Animals' (NIH Publication No 80-23, revised 1985). The animals were anesthetized by ether during the procedure and at the time of removal of the muscle for analysis. The EDL muscles of both leg limbs were dissected with exposure of proximal and distal tendons. The neurovascular trunks were sectioned at the entry of the muscle and the ischemia was completed by ligation with 5-0 Polypropylene sutures (Ethnor, Paris, France) without cutting the tendons as opposed to the original model of Carlson (3).

The EDL muscle of one leg was injected by using an Hamilton syringe with a specific sharp and flexible needle (N-50 type B, Ito Corp. Fuji, Japan) specifically chosen to minimize physical damage of muscle fibers), containing 100 μl of a solution of RGTA at a concentration of 50 μg/ml in phosphate buffered saline (PBS). The contralateral EDL muscle was injected with 100 μl of PBS and is referred to as 'control muscle or non-RGTA-treated muscle'. More precisely, in order to reduce muscle fiber damage and provide a homogenous repartition of RGTA, the injection was performed in two steps under a binocular microscope as follows: the needle was introduced into the mid-region of the EDL muscle at an angle so that it penetrated parallel to the muscle fibers toward the proximal tendon. As the syringe pushed the first 50 μl of the liquid into the muscle, the needle was slowly withdrawn. A second, identical 50 μl injection was performed with an opposite angle toward the distal tendon. Absence of leakage at the point of injection and through the epimysium as well as homogeneous distribution of the fluids were assessed in control experiments using fluorescent RGTA (not shown).

A first series of nine rats was used, each rat having both legs injected—one with RGTA, the other with PBS. Another series of six rats was injected into only one leg, three with RGTA and three with PBS. No differences were observed between the single and double injection procedures.

The EDL muscles were examined 7 days after injection. The muscles were removed by cutting both tendons beyond previous ligations, sectioned transversely into segments 6–7 mm long, and rapidly frozen in liquid isopentane cooled by liquid nitrogen at −150°C. Sample sections of 10 μm thick were made using a cryostat (Leica). Serial sections performed in the mid-, proximal, and distal regions were extemporaneously stained with Gomori's trichrome and examined with light microscopy. In both groups, muscle mean diameter, epimysium and peripheral zone thickness, mean diameter of the central ischemic area, and mean diameter of the myotubes were measured in the central part of the muscle under a 10× objective using a micrometric scale. The number of surviving muscle fibers in the peripheral zone was measured under a ×20 objective. Thirty different fields from both groups were randomly selected for the measurements.

RESULTS

As seen in **Fig. 1** and **Fig. 2**, in PBS-injected EDL muscle controls the peripheral zone contained few surviving cells and regenerating fibers. In these untreated muscles, a radial gradient of regenerating muscle fibers was established, with more mature fibers at the periphery of the muscle (Fig. 1). The EDL muscle treated by RGTA showed a thicker peripheral zone of surviving muscle fibers (**Fig. 3**) than controls. No transition between these surviving fibers and the neighboring regenerating zone (noted R, Fig. 3) could be detected.

Analysis of the histological sections of PBS- or RGTA-injected muscles (Fig. 1 and Fig. 3) indicate striking differences at the level of the epimysium, surviving muscle fibers and the regenerating central zone. The epimysium in non-RGTA-treated muscle

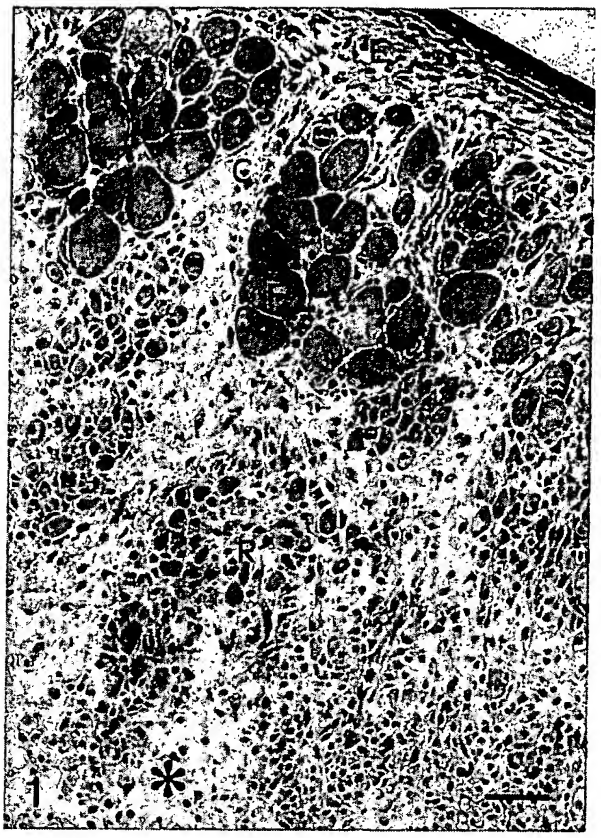

Figure 1 Low magnification of Gomori-stained transverse sections of the mid-proximal portion of non-RGTA-treated EDL from the center (*) to the epimysium (E) in the opposite region of the tendon. F indicates the surviving peripheral rim of muscle fibers. The central (regenerated) area contains myotubes or small regenerated myofibers (R). Magnification: ×80 (bar = 100 μm). Note significant thickening of the epimysium (E) and the fibrotic reaction (C) between the surviving muscle fibers (F). In the central (regenerated) region, a few small regenerated myotubes can be seen among proliferating mononucleated cells. Scarce vascularization and some remaining necrotic muscle fibers are detected.

(see E, Fig. 1) was constituted by a thick connective tissue containing several layers of fibroblastic cells that invaded the underlying muscle fibers (see C, Fig. 1). This was not detected in the RGTA-treated muscles (Fig. 3). In this case, the epimysium consisted of a very thin layer of connective tissue. No traces of invasion by connective tissue into the underlying muscle fibers or fibrosis in this zone could be detected.

After 7 days, the mean diameter in the central region of the untreated EDL muscle was 5.1 ± 0.2 mm and did not significantly differ from that of RGTA-treated muscles (5.2 ± 0.4 mm, **Table 1**). Inflammatory reaction in the epimysium was more pronounced in the non-RGTA-treated EDL, and the epimysium was more than sevenfold thicker than that of the epimysium from RGTA-treated muscles. PBS-injected EDL muscles were characterized by a large central area, where original muscle fibers had

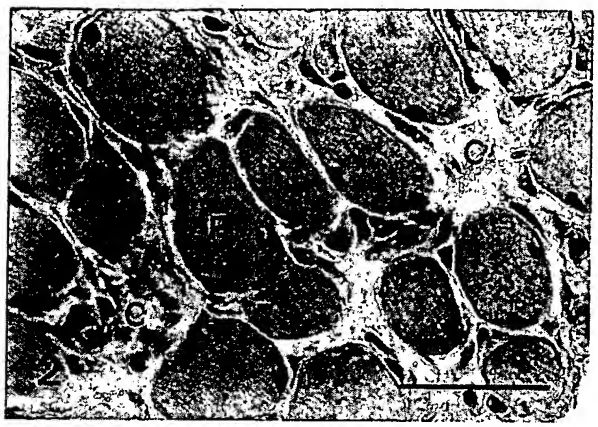

Figure 2. Gomori staining of transverse sections of non-RGTA-treated EDL muscle after 7 days of regeneration. Details of the remaining peripheral myofibers with development of the connective tissue (C).

completely disappeared, and by a thin peripheral zone containing an average of 3.5 ± 0.7 layers of surviving muscle fibers. RGTA-treated muscles were characterized by a reduced (20% smaller) degener-

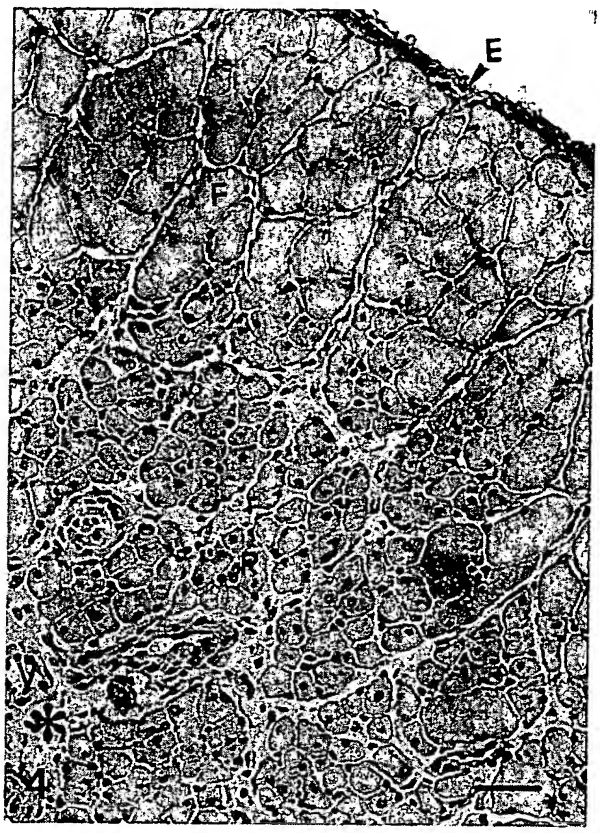

Figure 3. RGTA-treated EDL. Note normal thickness of the epimysium (E) and the increased number of surviving muscle fibers and layers without infiltration of connective tissue. The transition between the surviving zone (F) and the more central regenerating area is very sharp (see arrow). The regenerating zone shows numerous well-developed and distributed new fibers. The new fibers are already organized in bundles. Several blood vessels (V) are seen.

TABLE 1.

	Non-RGTA-treated EDL	RGTA-treated EDL	*P*
Epimysium thickness (μm)	87 ± 13	126	0.01
Peripheral zone thickness	280 ± 54	740 ± 35	0.05
Layers of surviving muscle fibers	3.5 ± 0.7	8.5 ± 1.9	0.05
(Regenerating) central zone diameter (μm)	4,470 ± 120	3,600 ± 150	0.05
Muscle diameter (mm)	5.1 ± 0.2	5.2 ± 0.4	ns
Mean diameter of the myotubes (μm)	8 ± 4	17 ± 5	
range (μm)	(6–21)	(13–26)	

n = 9 rats; P = statistical significance established using Student's t test; ns = nonsignificant.

ative central area and an enhanced (260% larger) peripheral zone containing 8.5 ± 1.9 layers of surviving muscle fibers. The mean diameter of the myotubes was 17 ± 5 μm (range 13 to 26) in the RGTA group and 8 ± 4 μm (range 6 to 21) in the control group. Dense connective tissue was present in large quantities among the fibers in the central region of the EDL muscle (Fig. 2) and was not visible in the RGTA-treated muscle (**Fig. 4**). The overall architecture in the central EDL was disorganized (**Fig. 5**) as opposed to a preserved internal structure (such as perimysium, noted by P in Fig. 4) induced by the RGTA treatment (**Fig. 6**).

Furthermore, in the ischemic muscle zone, blood vessels of the control EDL are located mainly in the epimysium and exhibit a 'sinusoidal' undifferentiated structure with large lumens (not shown). In EDL treated with RGTA, dense blood vessels are well differentiated (see V, Fig. 3 and 6) within the previously ischemic zone and highly ischemic environment.

On day 3, muscle fibers were invaded by macrophages phagocytosing the necrotic cytoplasm and the edema disappeared. On day 5, myoblasts and early myotubes were detected within the basal lamina of the original muscle fibers. On day 7, regeneration of new muscle fibers within the ischemic parts was observed, with only a small contribution from surviving peripheral muscle fibers. Ischemia induced by the ligation of each tendon and cutting of the neurovascular trunks passed through very similar phases. Indeed, at day 7, regeneration of new muscle fibers within the ischemic parts was also detected as well as two to four layers of surviving peripheral muscle fibers (Figs. 1, 2).

DISCUSSION

Sequential degenerative and regenerative phases occurring after free grafting of EDL muscle undergoing ischemia have been thoroughly characterized in the rat by Carlson (3) and in mdx mice by Anderson (23). Indeed, at day 1, the EDL muscle was placed in highly ischemic environment; as a result, all of the muscle fibers except for a rim 2–4 fibers thick around the periphery entered a state of ischemic necrosis.

A small increase of surviving fibers was observed

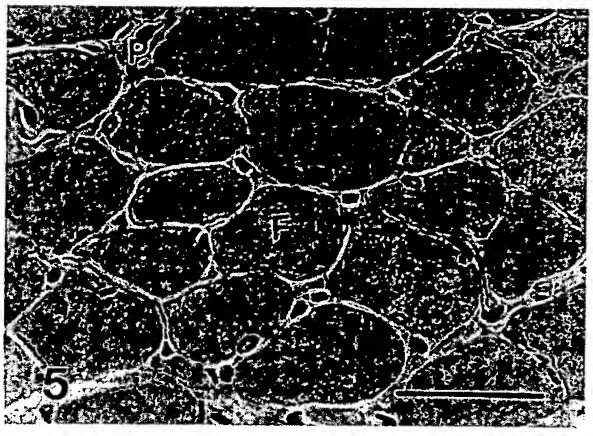

Figure 4. The absence of connective tissue is shown in RGTA-treated EDL. P = perimysium (bar = 50 μm, magnification: × 380). Treatment induced the preservation of muscle fibers, with small spaces between them and low-level development of connective tissue.

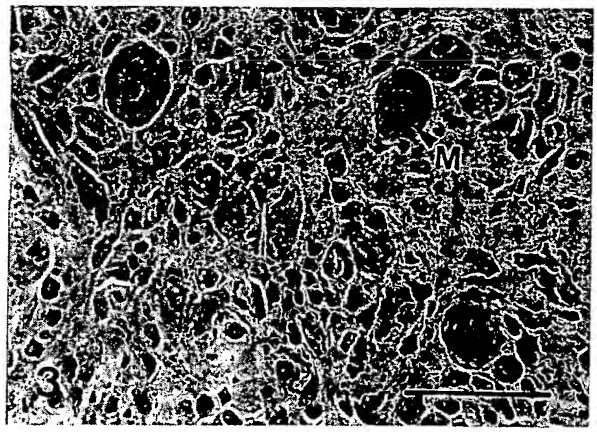

Figure 5. Gomori staining of transverse sections of non-RGTA-treated EDL muscle after 7 days of regeneration. Details of the ischemic central region, showing small regenerated myotubes (M) surrounded by many mononucleated cells (arrows).

Figure 6. Details of the ischemic central region containing many well-differentiated myotubes (M) (with central nuclei) in RGTA-treated EDL (bar = 50 m). Magnification: ×380. V = blood vessels. Treatment with RGTA accelerated and enhanced the differentiation of regenerated new fibers.

when compared with the whole muscle graft model. This difference is probably due to the fact that, in our model, the muscle is not subjected to changes of tension.

As presented in Results, treatment with a single injection of RGTA induced major differences compared with injured control muscle, whereas RGTA had no effect when injected into intact muscle. The absence or reduced fibrotic reaction at the level of the epimysium in the RGTA-treated muscle indicates that ischemia-induced necrosis was less effective at the muscle periphery and suggests a protective and control of the inflammatory effect by RGTA. In freely grafted EDL muscle, the fibrotic reaction was associated with revascularization from collaterals, which penetrated the epimysium and progressed in a centripetal way toward the center of the muscle (2). This protective effect was also observed in up to six to eight layers of peripheral muscle fibers. Comparative histological studies between intact and RGTA-treated ischemic EDL muscles show no significant differences at the level of the epimysium. The absence or inhibition of degradative enzymes released from dying and inflammatory cells could explain the maintenance of tissue integrity. RGTA was shown to inhibit elastase from plasmin and leukocytes (17, 18). Furthermore, RGTA protects HBGF(s) such as FGF 1, 2 or TGF-β1 from proteolysis (12, 14). Several growth factors are known to act as cellular or tissular survival agents. As in the case of muscle cells, a cardioprotection has been reported for FGF 2 and for insulin-like growth factor 1 (IGF1) when administered in an isolated rat heart model of ischemia perfusion (24, 25). RGTA may act either directly by protecting and potentiating HBGF(s) such as FGF 2 or indirectly by protecting binding proteins such as IGF BP3, 4, and 5, which also bind heparin. It is noteworthy that TGF-β has been shown to counteract the deleterious effects of tumor necrosis factor alpha and oxygen free radicals in reperfusion injury of myocardial ischemia (25). Protecting the bioavailability of TGF-β should also protect against free radical damage. In skeletal muscle regeneration, numerous studies have implicated growth factors, among which FGFs, IGF, and TGF-β are believed to play a prominent role. Similarly, in healthy tissues surrounding ischemic areas of skeletal muscle, a marked induction of FGF 2 mRNA has been reported (26).

We propose that RGTA may act as a survival and protective agent through the maintenance and protection of the bioavailability of preexisting and newly synthesized growth factors. In more central zones of the muscle, deprivation of oxygen is more important than at the periphery and the protective effect of RGTA on cellular integrity is no longer sufficient. Cellular necrosis occurs, followed by a strong inflammatory response. Growth factors may be released from their extracellular matrix compartment, and/or from necrotic vascular inflammatory cells as well as diffusing from neighboring healthy tissues (26). These factors are believed to participate in muscle regeneration. Indeed, growth factors such as FGFs, IGFI and II, and TGF-β can function individually or in combination to down- or up-regulate satellite cell proliferation and fusion, multiplication of cell lines in culture, in isolated single myofibers (27), and probably in muscle precursor cells *in vivo* (8). This complex regulation was also shown for hepatocyte growth factor (28), another HBGF, but not for FGF2 (29), although blocking antibody to FGF2 modifies muscle regeneration after injury (30). The basal lamina is a domain of the extracellular matrix in which FGFs are stored through their interaction with heparan sulfate (31). Thus, these structures can supply one of the growth factors controlling at least *in vitro* the proliferation of satellite cells (32). In mdx mice, which display persistent regeneration, the increasing rate of myoblast regeneration is correlated with a high level of FGF1 (33). TGF-βs are also stored in the extracellular matrix, and TGF-β1 and -3 have been shown to be expressed by regenerating muscle in the first day after trauma (30). We suggest that RGTA may act in this model by protecting the endogenously released HBGF as well as by inhibiting elastase, plasmin, and other degradation enzymes of inflammation (17, 18). The result of such action would be an enhancement of the bioavailability of local growth factors and a better preservation of the remaining muscle basal lamina.

A single injection of RGTA at the time of injury led to a striking enhancement of muscle fiber survival and regeneration of the ischemic muscle. At present, acute lower extremity ischemia results in high perioperative morbidity, with an amputation rate of

around 20% and mortality in the same range. As a result of this ischemia, nerves and muscles are the first structures to be degraded, leading to neuromuscular junction destruction, muscle fiber degeneration, and sarcolysis. This destruction is seldom followed by muscle regeneration and fibrotic tissue often replaces muscle cells, which may eventually degenerate to gangrene. Our results indicate that RGTA, assuming that they are devoid of the potentially carcinogenic benzylamide group, may represent a new class of therapeutic agents and a new strategy to preserve muscles from degeneration. FJ

The authors wish to thank Pr. Bruce M. Carlson (Wisconsin) and Dr. Margareth Buckingham for helpful suggestions and critical reading of this manuscript. We thank Arlette Duchesnay and Yolande Rosso for technical assistance. This work was supported by the Ministère de l'Education Nationale, the CNRS, Naturalia et Biologia and the Association Française contre les Myopathies.

REFERENCES

1. Carlson, B. M., and Faulkner, J. A. (1983) The regeneration of skeletal muscle fibers following injury: a review. *Med. Sci. Sports. Exercise* **15**, 187–198
2. Hansen-Smith, F. M., Carlson, B. M., and Irwin, K. L. (1980) Revascularization of the freely grafted extensor digitorum longus muscle in the rat. *Am. J. Anat.* **158**, 65–82
3. Carlson, B. M. (1975) Regeneration in free grafts of normal and denervated muscles in the rat: morphology and histochemistry. *Anat. Rec.* **183**, 47–62
4. Carlson, B. M., and Guttman, E. (1975) Regeneration in grafts of normal and denervated muscles in the rat: contractile properties. *Pfluegers Arch.* **353**, 215–225
5. Mauro, A. (1961) Satellite cells of skeletal muscle fibers. *J. Biophys. Biochem. Cytol.* **9**, 493–495
6. Schollmeyer, J. E. (1986) Possible role of calpain I and calpain II in differentiating muscle. *Exp. Cell Res.* **163**, 413–422
7. Gulati, A. K., Reddi, A. H., and Zalewsri, A. A. (1983) Changes in the basement membrane zone components during skeletal muscle fiber degeneration and regeneration. *J. Cell Biol.* **97**, 957–962
8. Husmann, I., Soulet, L., Gautron, J., Martelly, I., and Barritault, D. (1996) Growth factors in skeletal muscle regeneration. *Cytokine Growth Factor Rev.* **7**, 249–258
9. Larrain, J., Alvarez, J., Hassel, J. R., and Brandon, E. (1997) Expression of perlican, a proteoglycan that binds myogenic inhibitory basic fibroblast growth factor, is down regulated during skeletal muscle differentiation. *Exp. Cell Res.* **234**, 405–412
10. Meddahi, A., Blanquaert, F., Saffar, J. L., Colombier, M. L., Caruelle, J. P., Josefonvicz, J., and Barritault, D. (1994) New approaches to tissue regeneration and repair. *Pathol. Res. Pract.* **190**, 923–928.
11. Blanquaert, F., Saffar, J. L., Colombier, M. L., Carpentier, G., Barritault, D., and Caruelle, J. P. (1995) Heparan-like molecules induce the repair of skull defects. *Bone* **17**, 499–506
12. Meddahi, A., Benoit, J., Ayoub, N., Sezeur, A., and Barritault, D. (1996) Heparin-like polymers derived from dextran enhance colonic anastomosis resistance to leakage. *J. Biomed. Mater. Res.* **31**, 293–297
13. Fredj-Reygrobellet, D., Hriskova, D. L., Ettaïche, M., Meddahi, A., Josefonwicz, J., and Barritault, D. (1994) CMDBS, functional analogue of heparin sulfate as a new class of corneal ulcer healing agents. *Ophthalmic Res.* **26**, 325–331
14. Tardieu, M., Gamby, C., Avramoglou, T., Josefonvicz, J., and Barritault, D. (1992) Derivatized dextrans mimic heparin as stabilizers, potentiators, and protectors of acidic or basic FGF. *J. Cell. Physiol.* **150**, 94–203
15. Letourneur, D., Champion, J., Slaoui, F., and Josefoncicz, J. (1993) *In vitro* stimulation of human endothelial cells by derivatized dextrans. *In vitro Cell. Dev. Biol.* **29A**, 67–72
16. Desgranges, P., Barritault, D., Caruelle, J. P., and Tardieu, M. (1997) Transmural endothelialization of vascular prostheses is regulated in vitro by fibroblast growth factor 2 and heparan-like molecule. *Int. J. Artif. Organs* **20**, 589–598
17. Meddahi, A., Lemdjabar, H., Caruelle, J. P., Barritault, D., and Hornebeck, W. (1995) Inhibition by dextran derivatives of FGF-2 plasmin-mediated degradation. *Biochimie (Paris)* **77**, 703–706
18. Meddahi, A., Lemdjabar, H., Caruelle, J. P., Barritault, D., and Homebeck, W. (1996) FGF protection and inhibition of human neutrophil elastase by carboxymethyl benzylamide sulfonate dextran derivatives. *Int. J. Biol. Macromol.* **18**, 141–145
19. Gautron, J., Kedzia, C., Hussman, I., and Barritault, D. (1995) Injection of heparan-like substance in a crushed muscle accelerates its regeneration. *C. R. Acad. Sci. Paris* **318**, 671–376
20. Aamiri, A., Mobarek, A., Carpentier, G., Barritault, D., and Gautron, J. (1995) Effect of a substituted dextran on reinnervation during regeneration of adult rat skeletal muscle. *C. R. Acad. Sci. Paris* **318**, 1037–1043
21. Aamiri, A., Butter-Browne, G. S., Martelly, I., Barritault, D., and Gautron, J. (1995) Influence of a dextran derivative on myosin heavy chain expression during rat skeletal muscle regeneration. *Neurosci. Lett.* **201**, 243–246
22. Mauzac, M., and Josefonvicz, J. (1984) Anticoagulant activity of dextran derivatives. Part I: synthesis and characterization. *Biomaterials* **5**, 301–304
23. Anderson J. E. (1991) Dystrophic changes in mdx muscle regenerating from denervation and devascularization. *Muscle Nerve* **14**, 268–279
24. Buerke, M., Murohara, T., Skurke, C., Tomaselli, K., and Lefer, A. M. (1995) Cardioprotective effect of insulin like growth factor I in myocardial ischemia followed by reperfusion. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 8031–8035
25. Padua, R. R., Sethi, R., Dhallas, N. S., and Kardami, B. (1995) Basic fibroblast growth factor is cardioprotective in ischemia-reperfusion injury. *Mol. Cell Biochem.* **143**, 129–135
26. Walgenbach, K. J., Gratas, C., Shestak, K. C., and Becket, D. (1995) Ischemia-induced expression of bFGF in normal skeletal muscle: a potential paracrine mechanism for mediating angiogenesis in ischemic skeletal muscle. *Nature Med.* **2**, 453–459
27. Florini, J. A., and Magri, K. A. (1989) Effects of growth factors on myogenic differentiation. *Am. J. Physiol.* **125**, 701–711
28. Tatsumi, R., Anderson J. E., Nevoret, C. J., Halevy, O., and Allen, R. E. (1998) HGF/SF is present in normal adult skeletal muscle and is capable of activating satellite cells. *Dev. Biol.* **194**, 114–128.
29. Mitchell, C. A., McGeachie, J. K., and Grounds M. (1996) The exogenous administration of basic fibroblast growth factor to regenerating skeletal muscle in mice does not enhance the process of regeneration. *Growth Factors* **13**, 37–55.
30. Lefaucheur, J. P., and Sebille, A. (1995) Muscle regeneration following injury can be modified in vivo by immune neutralization of basic fibroblast growth factor, transforming growth factor β1 or insulin growth factor 1. *J. Neuroimmunol.* **57**, 85–91
31. Flaumenhaft, R., Moscatelli, D., Saksela, O., and Rifkin, D. B. (1990) Role of extracellular matrix in the action of basic fibroblast growth factor: matrix as a source of growth factor for long term stimulation of plasminogen activator production and DNA synthesis. *J. Cell. Physiol.* **140**, 75–81
32. Yamada, S., Buffinger, N., and DiMario, J. (1989) Fibroblast growth factor is stored in fiber extracellular matrix and plays a role in regulating muscle hypertrophy. *Med. Sci. Sports Exercise* **21**, S173–S180
33. Oliver, L., Raulais, D., and Vigny, M. (1992) Acidic fibroblast growth factor (aFGF) in developing normal and dystrophic (mdx) mouse muscles. Distribution in degenerating and regenerating mdx myofibers. *Growth Factors* **7**, 97–106

Received for publication June 16, 1998.
Revised for publication November 17, 1998.